



Host behaviour, age and sex correlate with ectoparasite prevalence and intensity in a colonial mammal, the little brown bat

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Abstract

The influence of behaviour on host-parasite dynamics has theoretical support but few empirical studies have examined this influence for wild-captured hosts, especially in colonial species, which are thought to face generally high risk of exposure. Behavioural tendencies of hosts in novel environments could mediate host exposure. We tested the hypothesis that behavioural tendencies of hosts, and host sex and age, correlate with prevalence and intensity of ectoparasites in a gregarious mammal, the little brown bat (*Myotis lucifugus*). We also tested whether relationships between host behaviour and parasite prevalence and intensity would vary between taxa of ectoparasites which differ in host-seeking behaviour. We predicted that individual hosts displaying active and explorative behaviours would have higher prevalence and intensity of parasites that depend on physical contact among hosts for transmission (mites) but that host behaviour would not influence prevalence and intensity of mobile ectoparasites with active host-seeking behaviour (fleas). We recorded behavioural responses of wild-captured bats in a novel-environment test and then sampled each individual for ectoparasites. After accounting for age and sex we found mixed support for our hypotheses in some but not all demographics. More active adult and young of the year (YOY) males were more likely to host mites while more active adult and YOY females were less likely to host fleas. Our results highlight possible differences in the influence of host and parasite behaviour on parasite transmission dynamics for colonial compared to non-colonial species and have conservation implications for understanding pathogen transmission in bat white-nose syndrome and other wildlife diseases.

Keywords

activity, exploration, Chiroptera, personality, infection, *Spinturnix*, *Myodopyslla*.

1. Introduction

Intersecting properties of hosts, parasites and shared environment influence host-parasite dynamics (Scholthof, 2006) and, within this context, transmission is a dynamic process that can be affected by host behaviour (Moore, 2002; Hawley et al., 2011). Identifying host behaviours that underlie transmission of parasites and pathogens (hereafter parasites) is important for predicting which individuals are most likely to transmit and/or acquire infections (Drewe, 2010). Since host behaviour can mediate parasite-risk, and parasites can negatively impact host fitness, understanding relationships between host behaviours and parasite exposure could provide insight into the ecology and evolution of host-pathogen dynamics (Moore, 2002).

Despite many benefits of coloniality, increased risk from parasites is a potentially significant cost which may increase with group size and host density (Alexander, 1974; Côté & Poulin, 1995) and fluctuate seasonally as hosts aggregate for mating, migration, or hibernation (Altizer et al., 2003). For example, seasonal changes in social aggregation by house finches (*Caprodacus mexicanus*) cause spatiotemporal variation in prevalence of avian mycoplasma (*Mycoplasma gallisepticum*) (Hosseini et al., 2004). Increased host density elevates the probability of contact among conspecifics which may be especially important for parasites and pathogens that require physical contact for successful transmission (Anderson & May, 1979; Altizer et al., 2003). In colonial hosts parasite risk should be uniform if all individuals interact equally (Anderson & May, 1992). However, in nature, even in colonial species with high contact rates among conspecifics, spatiotemporal aggregations of both hosts and parasites, and non-random contacts among individuals, predict variation in parasitism (Altizer et al., 2003). Individual behavioural tendencies may influence a variety of ecological and physiological traits (Sih et al., 2004) and are also thought to affect risk of parasitism for hosts (Barber & Dingemanse, 2010). However, despite implications of individual host behaviour for risk of parasitism, this relationship has received surprisingly little attention for wild populations (Kortet et al., 2010).

Macroparasites (i.e., multi-cellular species with low reproductive output, May & Anderson, 1979), including ectoparasites, rarely cause mortality of their hosts but can negatively impact energy reserves, body condition, and reproductive potential, reducing host fitness (Khoklova et al., 2002). Although intuitively appealing, the assumption that host behaviour influences risk of macroparasite exposure is largely theoretical and empirical data are available

from only a few model fish, bird and rodent species (e.g., *Lepomus gibbosus*, Wilson et al., 1993; *Parus major*, Dunn et al., 2011; *Rattus norvegicus*, Webster et al., 1994; Berdoy et al., 2000; *Tamias sibiricus*, Boyer et al., 2010; *Tamias striatus*, Patterson & Schulte-Hostedde, 2011). These studies largely support predictive models (Kortet et al., 2010) with active or explorative tendencies correlated with high parasite prevalence or intensity. All mammals studied in this context to date, however, occupy similar ecological niches and are characterized by similar social dynamics with primarily solitary individuals that infrequently come in contact with conspecifics (Boyer et al., 2010). Host-parasite dynamics could vary dramatically for colonial species.

Two general categories of ectoparasite transmission have been described in detail (Côté & Poulin, 1995). Contact or ‘contagious’ parasites require either direct physical contact or close spatial association of hosts for transmission (Côté & Poulin, 1995). In some bird species, mites require direct physical contact between hosts for transmission and cannot survive off their hosts (Proctor & Owens, 2000). Mobile parasites may actively search for hosts or move to locations where they are the most likely to encounter hosts such as oestrid flies that seek out caribou herds (*Rangifer tarandus*; Fauchald et al., 2007) or ixodid and argasid ticks which locate suitable hosts in their environment (Oliver, 1989). Like host behavioural tendencies, differences in patterns of host-seeking behaviour by parasites could affect prevalence and intensity of infection.

Temperate insectivorous bats have potential as model species to examine the influence of host and parasite behaviour, particularly in a colonial context. Female bats of many species congregate in maternity colonies numbering in the tens to hundreds of individuals. Maternity colonies provide warm temperatures in which mother bats raise their pups (Willis & Brigham, 2007) but, despite thermoregulatory benefits, adult females and young of the year (YOY) face high rates of ectoparasite exposure (Czenze & Broders, 2011). Variation in behaviour of ectoparasite taxa within colonies, combined with variation in host behaviour, could mediate parasite intensity and prevalence. Among the most common ectoparasites in maternity roosts of temperate bats are fleas and mites (e.g., Pearce & O’Shea, 2007). Fleas and mites have both been described as ‘contagious’ (Côté & Poulin, 1995) but they differ in terms of mobility. Mites complete their life cycle on the host and transmission requires physical contact between infected and susceptible bats (Christe et al., 2000). Vertical transmission occurs from adult females to pups, likely due

to close contact during lactation (Christe et al., 2000). In contrast to mites, only adult fleas parasitize bats and they are highly mobile, crawling on roost substrates toward potential hosts following a range of cues (Smith & Clay, 1988). Since fleas are so mobile, at any given time most of their population is thought to occur on substrate, rather than bats, resulting in potentially low infestation time but greater movement among hosts (Pearce & O'Shea, 2007). When maternity colonies disperse, both mites and fleas are transported to hibernacula by adult females and YOY (Czenze & Broders, 2011). Young of the year tend to be more heavily parasitized than adult females, possibly because of a naïve immunoresponse and less experience grooming (Christe et al., 2000).

In contrast to reproductive females, adult males and non-reproductive females of many bat species roost individually or in small groups, away from maternity colonies (Kunz & Lumsden, 2003) which should reduce risk of ectoparasitism for males (Christe et al., 2003, 2007). When maternity colonies disperse, bats congregate at hibernacula and engage in behaviour known as swarming (Fenton, 1969). Swarming appears to function primarily for mating and familiarizing YOY with potential hibernacula (Fenton, 1969). During swarming, bats fly in, through, and around hibernacula, frequently coming in contact with conspecifics during promiscuous mating (Thomas et al., 1979). Although mating contacts brief, the high frequency of contact between males and females, and bats and substrates in hibernacula, could have dramatic implications for transmission of ectoparasites throughout populations.

We conducted the first study to examine links between individual behavioural tendencies of hosts and the prevalence and intensity of ectoparasite infection in any colonial mammal. We used little brown bats (*Myotis lucifugus*) captured during fall swarming to test the hypotheses that ectoparasite prevalence and intensity is correlated with: (1) individual behaviour, sex and age of hosts; and (2) differences in host-seeking behaviour of different parasite taxa. We assessed individual behaviour using an open-field test designed to quantify activity and explorative tendencies of bats (Menzies et al., 2013). We tested four predictions of these hypotheses developed based on previous research from non-colonial animals. First, we predicted that female and YOY bats which were more active and explorative in the open field would exhibit higher prevalence and intensity of mites because transmission of mites requires direct contact between conspecifics and more active/explorative individuals should be more likely to come in contact with more conspecifics in

their roosts. Second, we predicted that open field responses would not correlate with prevalence and intensity of fleas because of the difficulty avoiding mobile, host-seeking ectoparasites. Third, we predicted that YOY males and females should exhibit similar patterns of ectoparasite infection and intensity because these groups presumably experience similar roosting conditions during development. Fourth, and finally, we predicted that adult males which were more active or explorative in the open field would have relatively high prevalence and intensity of both fleas and mites because these individuals should have higher contact rates with parasitized females, YOY bats, and substrates, during the fall swarm period.

2. Methods

2.1. Study sites

All procedures were approved by the University of Winnipeg Animal Care Committee, conducted in compliance with guidelines of the Canadian Council on Animal Care and approved under Manitoba Conservation Wildlife Scientific Permit number WB0612. Although all sites were negative for *Pseudogymnoascus destructans* (Pd), the fungal pathogen that causes white-nose syndrome (WNS), we followed U.S. Fish and Wildlife service guidelines for decontamination (United States Fish and Wildlife Service, 2012). We caught little brown bats between 17 July and 26 August 2012, at St. George Bat Cave near Fisher River First Nation, MB, Canada (51°26'N, 97°21'W), which houses approximately 11 000 bats in winter, as well as three caves near Grand Rapids, MB, Canada (53°12'N, 99°18'W) each of which houses 50–250 bats in winter. Results from both sampling locations were pooled for analyses because individuals from both locations experience similar conditions and belong to the same population (Martinez-Nunez, 2012; Norquay et al., 2013).

We caught swarming bats using harp traps placed at entrances to hibernacula. Upon capture, bats were held individually in disposable paper bags prior to behavioural trials and ectoparasite sampling (see below). Each bat was sexed, weighed with an electronic balance (± 0.1 g; Model HH 320, Ohaus, Parsippany, NJ, USA), and aged (adult or YOY) based on degree of ossification of the fifth metacarpal-phalangeal joint (Thomas et al., 1979). We measured forearm length (± 0.05 mm) and used the residuals of a linear regression between mass and forearm length as an index of body condition (BCI; Schulte-Hostedde et al., 2001).

2.2. *Open field test*

We quantified individual behaviour using an open field test modified from the hole-board test, which is commonly used to assess behaviour of rodents (File & Wardill, 1975; Martin & Réale, 2008) and which has recently been used to quantify behaviour of bats (Menzies et al., 2013). The test consisted of a rectangular arena (57 cm wide \times 42 cm tall \times 14 cm deep) made from opaque plastic with a transparent plexiglass cover and window screening on the back surface to facilitate climbing by the bats. Menzies et al. (2013) designed the test to be ecologically relevant for bats, eliciting behaviours that would occur when bats investigate potential roost openings in trees or other structures. The chamber was hung vertically so that bats entered from the bottom of the test and could climb up the flat vertical surface. Four blind holes (2 cm wide \times 1 cm deep) were drilled into this vertical surface to simulate roost openings bats might investigate in the wild. The two lower holes were positioned closer to the centre (i.e., interior holes, 15 cm from the edge and 10 cm from the bottom of the arena) and the two upper holes were positioned closer to the edges (i.e., peripheral holes, 5 cm from the edge and 5 cm from the top of the arena). Investigation of the holes closer to the center is thought to be associated with more exploratory behaviour in rodents and bats (Martin & Réale, 2008; Menzies et al., 2013). A start chamber (16 cm long \times 8 cm diameter tube) was fastened to the bottom of the arena. Bats were placed in the start chamber and given 60 s to enter the test arena voluntarily. A sliding door separating the main arena from the start tube was closed immediately after the bat entered the arena. If an individual did not enter within 60 s, it was gently pushed into the chamber using a plastic plunger.

We recorded all trials with a video camera (Sony AVCHD NightShot handycam HDR-XR550) and infrared lamp. To maintain natural conditions, all lights were switched off for the 10 min trial. After each trial the test and start chambers were cleaned thoroughly with alkyl based disinfectant (Lysol unscented wipes: United States Fish and Wildlife Service, 2012) and allowed to air dry for at least 10 min. To maximize sample size, we used two identical test chambers so that while one was being cleaned and dried the other could remain in use allowing us to measure more animals on a given night. We compared behavioural scores for bats run in each of the two arenas (see Section 2.4 below) and found no difference ($p > 0.35$) so results based on the two arenas were pooled. Behavioural responses in this open-field test

appear to meet the criteria for animal personality or temperament (Sih et al., 2004) because the test divides behaviours into virtually identical personality dimensions as those observed in previous studies of rodents, birds and fish, and because responses of individual bats were repeatable, at least over the short-term (i.e., 24 h; Menzies et al., 2013). However, because Menzies et al. (2013) were not able to confirm long-term repeatability of bats' responses in the test, and because we were only able to assess responses of each individual during a single test in this study, we do not use the term personality here and instead refer to open-field responses.

Videos were scored for a range of behaviours assessed in previous behavioural studies employing the hole-board test (e.g., Martin & Réale, 2008; Menzies et al., 2013). The length of time an individual spent in the start chamber before entering the main arena was recorded as latency to enter with maximum latency set at 60 s (Martin & Réale, 2008). Once bats were in the test we scored locomotion (time spent crawling), frequency of flight attempts, time spent echolocating (a stationary bat scanning the chamber with its mouth open), frequency of head dips (number of times an individual explored holes in the backboard), latency until the first head dip in a peripheral hole, latency until the first head dip in an interior hole, duration of grooming, and the number of fecal pellets produced during the trial. We also superimposed one vertical and one horizontal line, intersecting at the centre of the video image, to quantify locomotion as the number of times an individual crossed each line (i.e., line crossing). Vertical and horizontal line crosses were combined for analysis because they were highly correlated (Pearson $r = 0.59$).

2.3. Ectoparasite sampling

Ectoparasite prevalence and intensity were quantified immediately after completion of behavioural trials following methods described by Czenze & Broders (2011). First, the base and length of the internal and external pinna were examined, followed by the tragus. Second, wing and tail membranes were extended and examined on both dorsal and ventral sides. Third, the fur was examined by gently blowing on the dorsal and ventral surfaces of the torso which disturbed mobile ectoparasites making them highly conspicuous. This approach allowed us to readily quantify the prevalence and intensity of two species of ectoparasites. We identified ectoparasites based on the location of attachment and macroscopic diagnostic characteristics as described by Czenze & Broders (2011). Bat fleas (*Myodopsylla insignis*) were observed

crawling through the fur on the ventral and dorsal torso of infected bats. Wing mites (*Spinturnix americanus*) were observed crawling on, or attached to, the wing and tail membranes. Both *M. insignis* and *S. americanus* are ectoparasites that specialize on bats and have been reported on other hibernating bat species in North America (e.g., *Eptesicus fuscus*, Dick et al., 2003; *Myotis septentrionalis*, Czenze & Broders, 2011). However, in our study area, the little brown bat represents >96% of the hibernating bat population based on 25 years of capture data (over 10 000 captures) with <4% *M. septentrionalis* (M.A. Collis & C.K.R. Willis, unpublished data). *Eptesicus fuscus*, the only other hibernating bat species that might occur in Manitoba has never been captured in the province to our knowledge. Therefore, although possible, transmission via contact with other bat species is unlikely and little brown bats overwhelmingly dominate our study system.

2.4. Statistical analyses

All statistical analyses were conducted using R (version 2.15.0 GUI 1.51, R Development Core Team, 2012). We used principal component analysis (PCA; R function `prcomp`) to reduce the large number of behavioural variables into a smaller number of components for subsequent analysis. We scaled and centred the raw data by subtracting variable mean values from each value and dividing by the variable standard deviation. This resulted in a dataset with mean values of zero, ensuring that the first component described the most variance. We retained principal components that met the Kaiser–Guttman criterion (eigenvalues > 1; Kaiser, 1991). PC scores for each individual were used as predictor variables in subsequent analyses.

We used logistic regression to assess relationships between predictor variables and ectoparasite prevalence. We first tested for collinearity among predictor variables included in the initial global model (principal component scores, age (adult or YOY), sex, Julian day and BCI). We then used backwards stepwise elimination to remove the least significant term and re-evaluated models until only significant terms remained.

We used zero-inflated mixture models (R function `zeroinfl`) to assess the effect of our candidate variables on ectoparasite intensity. Zero-inflated mixture models are robust to zeroes in a dataset and can model relationships based on Poisson or negative binomial distributions (Zuur et al., 2009). We used the same predictor variables to assess intensity as for prevalence (see above) and selected a distribution parameter by comparing models generated

with either zero-inflated Poisson (ZIP) or zero-inflated negative binomial (ZINB) distributions (Zuur et al., 2009). We used Akaike's Information Criterion (AIC) to compare models and selected the distribution (ZIP or ZINB), which minimized AIC. We then determined effects of our predictor variables using backwards stepwise elimination (Zuur et al., 2009). We used AIC and likelihood ratio tests (LRTs) (in all cases AIC and LRT comparisons yielded identical results) to compare the fully saturated model against a series of alternative models each with a different variable removed. The variable with the highest p -value was identified and dropped from the fully saturated model (Zuur et al., 2009). Once this variable was dropped we then compared the new saturated model (i.e., with one less variable) to a subsequent series of alternative models. We repeated this process until only significant terms, or no terms, remained. We conducted separate analyses for each demographic (i.e., adult male, adult female, YOY male, YOY female) and both categories of ectoparasites. Values reported are those from the best fitting model. We assessed significance at $\alpha = 0.05$ and report all values as the mean \pm SD unless otherwise stated.

3. Results

We retained the first three principal components from our PCA of open field responses, which explained 56% of the total variance in the data (Table 1). The first component (PC1) was strongly associated with activity-based behaviours such as locomotion, flight attempts and number of line crosses so we interpreted PC1 as an index of activity. The second component (PC2) was strongly associated with exploratory behaviours such as total number of head dips and latency to head dip in peripheral holes so we interpreted PC2 as an index of exploration. The final component (PC3) was strongly associated with numbers of fecal pellets left in the arena after each trial, as well as latency to enter the arena so we interpreted PC3 as an index of anxiety.

Overall, 24% (40/167) of individuals sampled were parasitized by mites (Table 2) although prevalence was strongly influenced by demographic (Table 2: $\chi^2 = 27.7$, $p < 0.001$). In our initial model, sex ($F_{1,164} = 22.2$, $p < 0.001$) and age ($F_{1,164} = 6.07$, $p = 0.01$) were the most significant predictors of mite prevalence so we analysed each demographic separately. Similarly, 42% (69/167) of the bats we examined were parasitized by fleas and, again, this proportion was strongly influenced by demographic (Table 2:

Table 1.

Summary of results for principal component analysis of behavioural responses of little brown bats in a modified hole board test ($N = 167$).

Behavioural variable	PC1 (Activity)	PC2 (Exploration)	PC3 (Anxiety)
Line crossing	-0.54 ^a	-0.01	0.02
Locomotion	-0.51 ^a	-0.10	0.17
Flight attempts	-0.43 ^a	-0.02	-0.18
Echolocation	0.30	0.32	0.07
Number of head dips	-0.10	0.62 ^a	0.11
Latency head dip (inner holes)	-0.01	0.60 ^a	-0.19
Latency head dip (outer holes)	0.25	-0.25	0.18
Grooming	0.19	-0.06	-0.17
Latency to enter	0.13	0.03	-0.69 ^a
Pellets	-0.14	0.26	0.57 ^a
Standard deviation	1.67	1.31	1.06
% Total variation	27.9	17.3	11.3

PC1 indicates activity, PC2 indicates exploration and PC3 indicates anxiety. See 'Open field test' in Methods for description.

^a Variables that met the Kaiser–Guttman criterion.

$\chi^2 = 27.2$, $p < 0.001$). In our initial model, sex ($F_{1,162} = 10.0$, $p = 0.002$) and age ($F_{1,162} = 17.6$, $p < 0.001$) were the most important predictors of flea prevalence so we separated the dataset and analyzed each demographic separately. Mean intensity of mites was 0.44 ± 1.05 and, as for mite prevalence, it was strongly influenced by demographic (Table 2: $\chi^2 = 27.9$, $p < 0.001$). In our initial full model of mite intensity, sex was the most important predictor ($Z = -4.6$, $p < 0.001$). When we separated the dataset into males and females, age was not significant but to maintain consistency between prevalence and intensity analyses we analyzed each demographic separately. Mean flea intensity was 0.89 ± 1.75 and, as for flea prevalence, was strongly influenced by demographic (Table 2: $\chi^2 = 29.7$, $p < 0.001$) this time with age as the most significant predictor ($Z = 4.8$, $p < 0.001$). When we separated the dataset into adults and YOY sex was not significant but, again, to maintain consistency between prevalence and intensity analyses we analyzed each demographic separately.

3.1. Behaviour and mite parasitism

We predicted that mite prevalence and intensity would be higher or more active and explorative adult females and YOY. Mite prevalence on adult

Table 2.

Summary of flea and mite prevalence and intensity for 167 little brown bats (52 adult males, 37 adult females, 35 YOY males, 43 YOY females).

Demographic	Flea parasitism				Mite parasitism			
	Number parasitized	Number unparasitized	Parasitized intensity	Overall intensity	Number parasitized	Number unparasitized	Parasitized intensity	Overall intensity
Adult male	9	43	2.33 ± 1.41	0.40 ± 1.05	0	52	0	0
Adult female	14	23	1.07 ± 0.27	0.41 ± 0.55	15	22	1.87 ± 1.30	0.75 ± 1.23
YOY male	16	19	2.40 ± 2.69	1.06 ± 2.10	8	27	1.25 ± 0.46	0.28 ± 0.57
YOY female	30	13	2.57 ± 2.46	1.79 ± 2.36	17	26	1.81 ± 1.60	0.84 ± 1.53
Overall	69	98	2.17 ± 2.16	0.90 ± 1.75	40	127	1.85 ± 1.44	0.44 ± 1.06

Mean intensity (\pm SD) is displayed for only the parasitized bats (parasitized intensity), as well as for all bats in that demographic (overall intensity) including individuals that were not parasitized (i.e., intensity = 0).

Table 3.

Summary of logistic regression model decomposition for adult females, YOY females, YOY males and adult males assessing the effect of five explanatory variables on the prevalence of fleas and mites.

Variable	Flea parasitism			Mite parasitism		
	<i>F</i>	df	<i>p</i>	<i>F</i>	df	<i>p</i>
Adult females						
PC1	7.9	1, 34	0.008	0.004	1, 32	0.95
PC2	0.08	1, 31	0.77	0.12	1, 34	0.72
PC3	1.4	1, 32	0.24	0.14	1, 35	0.71
BCI	3.2	1, 33	0.08	0.0005	1, 31	0.98
Date	5.4	1, 34	0.03	0.1	1, 33	0.75
YOY females						
PC1	8.7	1, 40	0.006	3.2	1, 40	0.08
PC2	4.3	1, 37	0.04	0.75	1, 39	0.39
PC3	0.04	1, 38	0.85	0.31	1, 38	0.57
BCI	0.43	1, 40	0.52	4.5	1, 41	0.04
Date	0.58	1, 39	0.45	0.005	1, 37	0.94
YOY males						
PC1	0.01	1, 31	0.91	2.1	1, 32	0.16
PC2	0.004	1, 29	0.95	4.1	1, 33	0.07
PC3	0.34	1, 32	0.53	0.08	1, 30	0.77
BCI	0.009	1, 30	0.93	0.004	1, 29	0.95
Date	0.91	1, 33	0.34	0.93	1, 31	0.34
Adult males						
PC1	0.01	1, 47	0.91	N/A	N/A	N/A
PC2	0.05	1, 48	0.83	N/A	N/A	N/A
PC3	0.003	1, 46	0.95	N/A	N/A	N/A
BCI	1.06	1, 49	0.31	N/A	N/A	N/A
Date	5.8	1, 50	0.02	N/A	N/A	N/A

females was 41% (15/37) and was not influenced by any of our predictor variables (Table 3). Intensity of mites on adult females was 0.75 ± 1.23 and as for prevalence, none of our predictor variables influenced mite intensity (Table 4). We also expected that mite parasitism would be similar on YOY males and females. Mite prevalence on YOY females was 39% (17/43) and BCI positively predicted mite prevalence, while none of our behavioural scores (Table 3) or date were predictors. Mite intensity on YOY females was 0.84 ± 1.53 and more active bats hosted more mites (Table 4). BCI was associated with mite intensity but none of our other behavioural variables or date were significant predictors. Similarly, mite prevalence on YOY males

Table 4.

Summary of the best choice zero-inflated mixture models selected by AIC and LRT comparisons of ectoparasite (flea and mite) intensity for little brown bats.

	Estimate	SE	z-value	p-value
Adult females–Flea parasitism				
Intercept	−1.07	0.30	−3.59	<0.001
PC1	−0.34	0.17	−2.01	0.04
Adult females–Mite parasitism				
Intercept	0.29	0.26	1.08	0.28
PC3	0.31	0.18	1.67	0.09
YOY females–Flea parasitism				
Intercept	0.45	0.17	2.73	0.01
PC1	−0.36	0.12	−3.10	0.002
YOY females–Mite parasitism				
Intercept	−0.22	0.26	−0.84	0.40
PC1	0.38	0.20	1.91	0.05
BCI	0.79	0.41	1.94	0.05
YOY males–Flea parasitism				
Intercept	1.48	0.88	1.68	0.09
Day	−0.05	0.03	−1.74	0.08
YOY males–Mite parasitism				
Intercept	−1.58	0.76	−2.08	0.04
PC2	0.84	0.43	1.96	0.05
Adult males–Flea parasitism				
Intercept	−2.30	0.91	−2.53	0.01
Day	0.09	0.03	3.50	<0.001

was 22% (8/35) and, as for females, none of our behavioural scores, BCI, or date were predictors (Table 3). Mite intensity on YOY males was 0.28 ± 0.57 and was associated with exploration, while neither of our other behavioural variables, BCI, or date were significant predictors (Table 4).

3.2. Behaviour and flea parasitism

We predicted no relationship between behavioural scores and flea prevalence or intensity for adult females and YOY. Flea prevalence on adult females was 38% (14/37) and probability of flea parasitism was lower for more active individuals with higher values for PC1 (Figure 1) but was not related to other behavioural variables or BCI (Table 3). Flea prevalence on adult females also increased as the swarm period progressed (Table 3). Intensity of fleas on adult females was 0.41 ± 0.55 and more active bats hosted more fleas

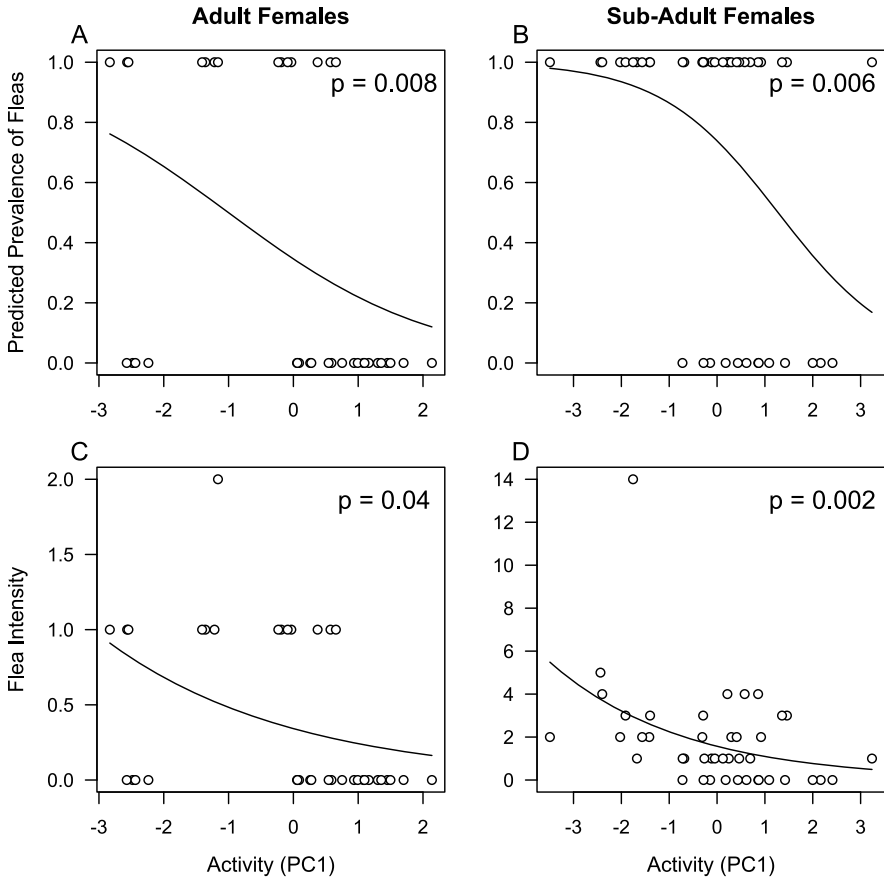


Figure 1. Probability of flea parasitism generated using best-fit logistic regression models for adult females (A) and young of the year (Sub-Adult) females (B) as a function of the activity (PC1); Predicted intensity of flea parasitism generated using best-fit zero-inflated mixture models for adult females (C) and YOY females (D) as a function of activity.

(Figure 1), while other behavioural variables, BCI, or date were significant predictors (Table 4). We also expected that flea parasitism would be similar on YOY males and females. Flea prevalence on YOY females was 70% (30/43) and, as for adult females, probability of flea parasitism was lower for more active individuals with higher scores for PC1 (Figure 1). However, flea prevalence was higher for more explorative individuals with higher scores for PC2, while PC3, BCI, and date were not predictors (Table 3). Flea intensity on YOY females was 1.79 ± 2.36 and as for adults, more active bats hosted more fleas (Figure 1). None of our other behavioural variables, BCI or date

predicted flea intensity for YOY females (Table 4). Flea prevalence on YOY males was 46% (16/35) and was not predicted by any behavioural scores, BCI, or date (Table 3). Flea intensity on YOY males was 1.06 ± 2.10 and was weakly associated with date but no other behavioural variables or BCI (Table 4).

3.3. Behaviour and parasitism for adult males

Flea prevalence on adult males was 17% (9/52) and mean intensity was 0.40 ± 1.05 and, as in adult females, increased as the swarming period progressed (Table 3). However, none of the behavioural variables, or BCI, were related to flea intensity for adult males (Table 4). No adult males were parasitized by mites so we were unable to perform an analysis of prevalence or intensity for this group.

4. Discussion

We detected correlations between behaviour and parasite load that were consistent with previous studies of non-colonial hosts for some demographics but strikingly different for others (Boyer et al., 2010; Patterson & Schulte-Hostedde, 2011). The influence of host behaviour also varied depending on the pattern of host-seeking behaviour of ectoparasites. For adult and YOY females we found consistently high prevalence and intensity of contact ectoparasites (i.e., mites) and no influence of host behaviour. However, we found a negative association between activity and the prevalence and intensity of host-seeking ectoparasites (i.e., fleas). By contrast, adult and YOY males had consistently low prevalence and intensity of ectoparasites with no effect of individual behaviour on prevalence or intensity of either category of ectoparasites. Taken together, these findings highlight the combined importance of age and sex of hosts, and host-seeking behaviour of parasites on transmission and suggest that predictions about parasite dynamics based on patterns for non-colonial species do not necessarily explain patterns of prevalence or intensity in a colonial context.

Our first prediction was that active females and YOY would be more likely to host mites because these individuals would presumably come into contact with conspecifics more frequently in the maternity colony, increasing their exposure. We found no support for this prediction in any demographic and surprisingly low prevalence and intensity of mites overall (Table 1). Mites may be relatively easy to groom compared to fleas because of their high

host affinity and relative immobility (Czenze & Broders, 2011). Thus, mite prevalence and intensity at swarming sites may not reflect patterns at maternity colonies where exposure for adult females and YOY should be high. Consistent with this hypothesis, in bent-winged bats (*Miniopterus shreibersii*), mite prevalence at maternity colonies was twice as high as at swarming sites (Lourenço & Palmeirim, 2007). This absence of a relationship between mite parasitism and host behaviour suggests that host activity and exploration have less overall influence on host-parasite dynamics than for non-colonial species. This could partly reflect seasonality of parasite risk and parasite life cycles, combined with high grooming potential for mites, mitigating potential influence of host behaviour. It would be interesting to revisit this aspect of the study at a maternity colony where prevalence and intensity of mites may be higher.

Our second prediction was that host behaviour of adult females and YOY would not be related to flea parasitism because mobile, host-seeking parasites should be difficult for all hosts to avoid in maternity roosts. In contrast, this time we did detect a relationship between host behavioural tendencies and flea prevalence and intensity. Relatively inactive adult and YOY females, but not YOY males, were the most likely to host fleas. This could reflect a combined effect of host and parasite behaviour at maternity colonies and host behaviour at swarming sites. Inactive adult and YOY females may be easier for host-seeking fleas to locate and parasitize within colonial maternity roosts which likely accounts for part of this effect. During the swarming period, however, a high proportion of the flea population lives on the substrate and thus fleas may behave more like contagious ectoparasites at this time of year, presumably spreading among individuals as a result of mating behaviour. Again these results suggest that predictions about relationships between host behaviour and parasite risk based on non-colonial species may not reflect patterns in a colonial context.

A couple of scenarios could explain the higher observed intensity and prevalence for females. First, if inactive females mate with fewer males they may exhibit higher prevalence and intensity because they retain adult fleas initially obtained in maternity colonies, rather than spreading them to mates. Second, although prevalence and intensity of fleas was lower for males than females, males will still host some fleas (Figure 1) and have potential to spread them back to females. Thus, if inactive females come in contact with fewer males during swarming this would further reduce their risk of flea

parasitism. Host behaviour mediates mating encounters in a variety of taxa. In common lizards (*Lacerta vivipara*) social females were more likely to be subject to mating attempts by males (Cote et al., 2008). Similarly, bold female bighorn sheep (*Ovis canadensis*) reproduced earlier and more often than shy females (Réale et al., 2000).

Our third prediction was that YOY males and YOY females would exhibit similar prevalence and intensity for both fleas and mites because of shared conditions in maternity colonies (e.g., Lourenço & Palmeirim, 2007). In contrast, the lower rate of intensity and prevalence we observed for YOY males suggests that YOY may behave more like adults of the same sex sooner than previously thought. For example, during the swarm period YOY males may begin roosting alone or in smaller groups like adult males, reducing their risk of exposure, while YOY females maintain a colonial lifestyle. In other vertebrate species, behavioural differences between males and females can also regulate parasite exposure. In great tits, males exhibit better problem-solving ability in standardized tests than females, presumably because of a more diverse diet and foraging behaviour, which results in increased exposure to malaria vectors during foraging (Dunn et al., 2011). We recommend that future studies quantify behavioural tendencies of juveniles and YOYs at maternity colonies, and if possible, repeat these tests at maternity colonies and swarming sites to determine the influence of behaviour during the post-weaning phase on host-parasite dynamics.

Our fourth prediction was that active or explorative males would have high prevalence and intensity of both fleas and mites because active, explorative males would be more likely to encounter parasitized conspecifics and substrates during swarming. We found little support for this prediction, possibly because so few adult males were parasitized by fleas and no adult males were parasitized by mites. Even though adult males (and potentially YOY males once they disperse from maternity colonies) are relatively solitary, ectoparasite transmission should still occur at least at low levels at swarming sites. In ring-necked pheasants (*Phasianus colchicus*), males are relatively solitary and were only exposed to lice during breeding when lice were transmitted from heavily infested females (Hillgarth, 1996). Our data could reflect a similar trend for little brown bats. With a larger sample of infected males we might have detected this relationship and we suggest that future studies quantify mating encounters of different individual males to shed light on the role of mating in transmission.

Consistent with previous studies of bats, but in stark contrast to studies of non-colonial species, we found that ectoparasite prevalence and intensity were greater in females and YOY's compared to adult males (Czenze & Broders, 2011). Inter-demographic variation in parasite prevalence and intensity can result from variation in behaviour between demographics, which is regulated by sex- or age-specific ecological/life history traits. In northern flying squirrels (*Glaucomys sabrinus*), prevalence of ectoparasites in males (90%) was dramatically higher than that of females (12%), which likely reflects male-biased dispersal (Perez-Orella & Schulte-Hostedde, 2005). In another solitary mammal, the Siberian chipmunk, adult males had larger home ranges than females and YOY, resulting in greater prevalence and intensity of ticks (Boyer et al., 2010). Our results demonstrating the opposite sex bias highlights the strong influence of female-biased social behaviour and coloniality for bats. Parasite risk is thought to be a major cost of social behaviour (Alexander, 1974; Altizer et al., 2003) and sociability is a trait with potentially important implications for parasite transmission. Despite the relatively solitary nature of adult male bats, they are still more gregarious than many other mammalian species (Thomas et al., 1979) and we recommend that future studies quantify individual differences in sociability among demographics and individual bats to assess its importance for transmission (e.g., Kilgour & Brigham, 2013).

An underlying assumption of our analysis is that individual behavioural tendencies affected ectoparasite infection, rather than ectoparasite infection influencing behaviour. In the absence of experiments manipulating ectoparasite prevalence and intensity, however, we cannot exclude the possibility that ectoparasites affected the host behaviours we measured. Separating cause from effect in studies linking behaviour and host-parasite dynamics is difficult (Barger & Dingemans, 2010) and parasite infection can influence behaviours like habitat use and risk taking (e.g., *Toxoplasmosis*: Berdoy et al., 2000). However, in our case, the behaviour most likely to be influenced by parasite prevalence or intensity was grooming and we found no evidence of this relationship. Previous studies have adopted a similar causal explanation for correlations between parasites and host behaviour (e.g., Boyer et al., 2010) but we suggest future experiments which manipulate parasite loads experimentally to tease out cause from effect (e.g., Giorgi et al., 2004).

Our results may be useful for understanding emerging infectious diseases and zoonotic pathogen dynamics in bats (e.g., Frick et al., 2010; Luis et

al., 2013) and have implications for conservation behaviour (Angeloni et al., 2008). Emerging infectious diseases are threatening a variety of wildlife species with extinction (McCallum, 2012) and understanding the role of host behaviour in pathogen transmission could provide crucial conservation information. Our results are potentially valuable for understanding the influence of host behaviour on spread of contact pathogens in social species (e.g., sylvatic plague in prairie dogs, Cully & Williams, 2001; white-nose syndrome (WNS) in bats, Frick et al., 2010; Lorch et al., 2011). Our results suggest that transmission of contact parasites or pathogens are mediated by host behaviour and demography and that transmission will be especially high for the most active members of colonial species. For example, if activity mediates numbers of mating encounters for females, and thus contact rates between females and males at swarming sites, host behavioural traits could influence the spread of WNS.

Our study supports the hypotheses that, despite high contact rates during both summer (especially for females and) and autumn (for all demographics), variation in ectoparasite prevalence and intensity is mediated by host behaviour, age, and sex in a highly gregarious mammal. Previous studies examining the role of host behaviour have focused on non-colonial vertebrates and, while some of the patterns we observed were similar, the gregarious nature of bats appears to result in different patterns of prevalence and intensity. Incorporating sociality as a potential explanatory behaviour in parasite studies will be useful for gaining a full understanding of parasite transmission dynamics in these species.

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